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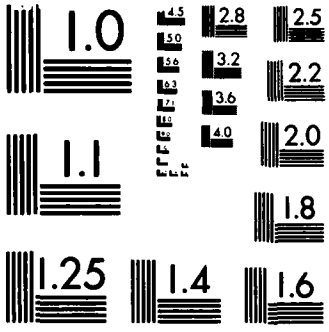

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In the past year we have established a new laboratory for the determination of macromolecular structure. Currently, facilities are in place for data collection, data processing, molecular modeling and x-ray refinement of structures of up to 100,000 molecular weight in their crystallographic asymmetric unit. In parallel with establishing a new laboratory, we have pursued structure investigations of hemoglobin

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from the sea lamprey, aspartate carbamoyltransferase from Escherichia coli and p-nitrobenzylidine aminoguanidine, a small molecule which is an acceptor of the adenosine diphosphate ribosyl group in an enzyme mediated reaction. In addition to the structural studies above we have made a theoretical study by techniques of energy minimization of possible modes of aggregation of lamprey hemoglobin and the relationship between aggregate formation and cooperativity expressed in solutions by lamprey hemoglobin.

Final Report, October 1985

Monies from the NIH, ONR, Iowa State University and the Petroleum Research Fund have equipped and financed the operation of a laboratory for the structure determination of macromolecules. The items of equipment currently installed and in operation are, 1) two x-ray generators, 2) one computer controlled diffractometer, 3) one precession camera, 4) one combined precession oscillation camera, 5) one water refrigeration unit, 6) two x-ray tubes and 7) a color graphics system. One of the two generators is a source for x-rays for the diffractometer. The generator is of high stability and incorporates the latest technology in solid state transformers for demands of total power up to 3 kilowatts. The second generator uses the old technology and is, in fact, a refurbished generator. The stability of the oil transformer is an order of magnitude less than that of the solid state generator. As a consequence, the refurbished generator will be employed in film data collection, where stability does not have an impact on the quality of data. All machined equipment is of the highest quality, particularly in regard to the x-ray diffractometer. The high standards for machined parts and specific design features of the diffractometer will simplify the process of optical alignment of the instrument. Furthermore, in our consideration of equipment, we gave significant weight to anticipated expenses in maintenance. We decided against the purchase of components which had poor service records and which were not essential to the productivity and quality of research. In particular there is no disk storage system associated with the diffractometer, all data are output to magnetic tape or stored in computer memory. In addition to the hardware above, we have purchased an Evans and Sutherland PS-300 color graphics system for molecular modeling. All sources of revenue for equipment total $180,000, $50,000 of which comes from the ONR.

Work on hemoglobin from the sea lamprey was begun at the Naval Research Laboratory in 1984 and continued, here, at Iowa State University in collaboration with Wayne A. Hendrickson (Columbia University) and Warner Love (Johns Hopkins University). Presently, a molecular model for the protein and ambient solvent of the complex of cyanide with methemoglobin V from the sea lamprey Petromyzon marinus yields an R-factor of 0.142 against X-ray diffraction data to 2.0 Å resolution. The root-mean-square discrepancies from ideal bond length and angle are, respectively, 0.014 Å and 1.5°. Atoms that belong to planar groups deviate by 0.012 Å from planes determined by a least-squares procedure. The average standard deviation for chiral volumes, peptide torsion angle and torsion angles of side-chains are 0.150 $Å^3$, 2.0° and 19.4°, respectively. The root-mean-square variation in the thermal parameters of bonded atoms of the polypeptide backbone is 1.21 $Å^2$, the variation in thermal parameters for side-chain atoms is 2.13 $Å^2$. The model includes multiple conformations for 11 side-chains of the 149 amino acid residues of the protein. We identify 231 locations as sites of water molecules in full or partial occupancy. The sum of occupancy factors for these sites is approximately 154, representing 28% of the 550 molecules of water within the crystallographic asymmetric unit.

The environment of the heme in the cyanide complex of lamprey methemoglobin resembles the deoxy state of the mammalian tetramer. In particular, the bond between atom NE2 of the proximal histidine and the Fe lies 5.1° from the normal of the heme plane. In deoxy- and carbonmonoxyhemoglobins, the deviations from the normal to the heme plane are 7 to 8° and 1°, respectively. Furthermore, the inequality in the distance of atom CD2 of the proximal histidine from the pyrrole nitrogen of ring-C of the heme (distance = 3.29 A) and CE1 from the pyrrole nitrogen of ring-A (distance = 3.06 A) is characteristic of deoxyhemoglobin, not carbonmonoxyhemoglobin, where these distances are equal. Finally, a hydrogen bond exists between carbonyl 111 and the hydroxyl of tyrosine 149. The corresponding hydrogen link in the mammalian tetramer is central to the T to R state transition and is present in deoxyhemoglobin but absent in carbonmonoxyhemoglobin. We suggest that the low affinity of oxygen for lamprey hemoglobin may be a consequence of these T-state geometries.

As an extension of the recent refinement work on lamprey hemoglobin we have modeled the putative dimeric state of lamprey hemoglobin. Dimers of lamprey hemoglobin exhibit cooperativity in the uptake of oxygen. Modeling studies have shown two likely modes of association of monomers of lamprey hemoglobin. One mode of association is an analogue of the cooperative interface of mammalian globins, the $\alpha_1\beta_2$ interface. The $\alpha_1\beta_2$ model for the lamprey dimer after energy minimization affords a structurally feasible network of intermolecular hydrogen bonds between aspartate 112 and glutamine 114 of one molecule of lamprey hemoglobin to tyrosine 115 and glutamine 114 of the second molecule. A hydrogen bond between glutamine 110 and glutamate 50 has poor geometry, but may be possible for dimers in the reduced state. Equally plausible for lamprey hemoglobin is an interaction between the E and F helices, an association of monomers observed for clam hemoglobin. The E-F model for the lamprey dimer indicates hydrogen bonds/salt-links are present between glutamate 31 and lysine 93, arginine 71 and aspartate 83, tyrosine 30 and aspartate 83 and glutamate 75 and asparagine 79. A salt-link between arginine 76 and the propionate side chain of ring A of the heme is possible also, but only after adjusting the torsion angles of both functional groups. Unlike the $\alpha_1\beta_2$ dimer of lamprey hemoglobin, however, the E-F dimer has an unfavorable electrostatic interaction between lysine 97 and arginine 71. As lamprey hemoglobin represents a point of divergence in the evolution of hemoglobins between mammals and molluscs, we suggest both the E-F and $\alpha_1\beta_2$ modes of association are possible. Future studies of lamprey hemoglobin could reveal much concerning the energetics of cooperativity in mammalian hemoglobins as the process of ligand binding in lamprey hemoglobin can be decoupled from the mechanism of cooperativity.

In support of the modeling studies of dimers of lamprey hemoglobin, we developed a new computer program for the superposition of protein structure. The program calculates a coordinate transformation which minimizes the root-mean-square deviation between atoms representing homologous structure in the two proteins. All atoms of the main chain and those atoms of side chains which bear common atomic labels contribute to the calculation of the transformation. Required input by the user is either a small set of integers representing the sequence numbers of homologous residues in the two proteins and/or the initial and terminal residues of homologous elements of secondary

structure. After using the starting set of homologies to calculate an initial transformation, the program discards the user input and then determines the full set of homologous residues by application of simple criteria. The superposition which results, is the point of departure of a search for alternate transformations which represent superpositions of merit. The computation time for the superposition of two structures of 150 residues is approximately 30 seconds on a VAX 11/780 and rises linearly with the size of the problem. Thus, the program is inexpensive in computer time and applicable to even the largest of macromolecules whose three dimensional structures are known.

In support of research efforts of Dr. D. Graves, Iowa State University, Department of Biochemistry, we have determined the structure of the small molecule p-nitrobenzylidine aminoguanidine (NBAG). NBAG is an acceptor of the adenosine diphosphate ribosyl group in enzyme mediated reactions central to the action of cholera toxin. Soman (Soman, G., Tomer, K. B. and Graves, D. J. (1983) Anal. Biochem. 134, 101-110) assays for the activity of adenosine diphosphate ribosyl transferases by using NBAG as an acceptor of the adenosine diphosphate ribosyl group. The kinetics of the transfer reaction could be sensitive to the tautomeric form of the guanidinium group as well as to its state of protonation. Structures of NBAG under conditions of high and low pH will provide information relevant to current speculation on the mechanism of transfer reactions. We have completed the structure determination of NBAG in a low pH form and are currently working on the structure of the high pH form.

Refinement work of aspartate carbamoyltransferase from Escherichia coli is in collaboration with William N. Lipscomb of Harvard University. Efforts over the past six months have decreased the R-factor from 0.22 to 0.18 at 2.6 Å resolution. The refined model reflects significant revisions in an earlier interpretation of the regulatory chain of the enzyme. The phosphates of CTP bind to lysines 56 and 94 of the regulatory chain. Earlier interpretations have lysine 60 at the phosphate binding site. Interactions between the protein and base of CTP are evident in electron density maps. However, assignment of amino acids to the density must await further refinement and interpretation.

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